REMARKS

With entry of this amendment, claims 1 - 143 are pending in the Application. By this amendment, claims 6, 8, 11, 16, 19, 48, 52, 59, 68, 91, 110, 132, 134, 136, 140, 141 and 143 have been amended for clarity in accordance with the Examiner's suggestions. All of the amendments presented herein are fully supported by the specification and no new matter has been added to the application. Entry of this amendment is respectfully requested.

Election/Restriction

Applicants acknowledge that the Office has reconsidered and withdrawn the species election requirement set forth in the Office communication dated April 23, 1999.

Claim Objections

Claims 6 – 9, 17, 55 and 68 are objected to under 37 CFR 1.75(c), as being of improper dependent form for allegedly failing to further limit the subject matter of a previous claim.

With regard to claims 6-9, the Office asserts that these claims expand the subject matter of the parent claim, "because RSV and measles are not `heterologous PIV sequence'" as required by the parent claim." To resolve the Office's concerns, Applicants have amended claims 6 and 8 herein to specify that the chimeric PIV incorporating a heterologous PIV sequence "further incorporates" a heterologous sequence from RSV or measles. As originally intended, these claims are directed to chimeric PIV incorporating nucleotide sequences from more than one PIV within the genome or antigenome which are further modified as vectors for incorporation of heterologous sequences, e.g., sequences encoding protein or peptide antigens, of non-PIV pathogens such as measles or RSV.

Concerning claims 17 and 68, the Office considers that the subject claims fail to limit the parent claims, on the basis that multiple mutations can be engineered at a "position

corresponding to" an identified mutant residue within the exemplary biologically derived mutant virus JS cp45. The latter point is correct. For example, as described in the specification, conservative substitutions and even non-conservative changes at a site of mutation identified within JS cp45 are contemplated within the invention to yield attenuated recombinant vaccine candidates. Accordingly, the dependency of the subject claims has been amended (directly in claim 68 and by amendment of claim 16 in the case of claim 17) consistent with the Office's suggestion to avoid the perceived conflict of scope.

With respect to claim 55, Applicants respectfully submit that there is not a conflict of scope as suggested by the Office. The base claim 52 only requires that an expression vector be included in the cell or cell-free system that comprises "one or more polynucleotide molecules encoding N, P, and L proteins." This composition does not exclude further addition of a coinfected PIV within more detailed embodiments to supply at least one of the recited N, P, and L proteins, irrespective of whether the terms "expression vector" and "coinfection with PIV" are exclusive or related.

Patentability Under 35 U.S.C. 112, Second Paragraph

Claims 15, 16, 19, 30, 36, 37, 39, 65-69, 71, 72, 78, 81, 82, 105, 106, 109-111, 113, 114, 127, 128, 130-136, 138-141 and 143 are rejected under 35 U.S.C. 112, second paragraph, as allegedly indefinite.

Concerning claims 19 and 110, the dependency of these claims has been amended for clarity in accordance with the Office's suggestions.

With respect to claims 132, 134, 136, 140, 141, and 143, the Office submits that these claims are confusing in requiring "a full complement" of mutations present in JS cp45 while referring to a "full complement" as a list of specific mutations. The subject claims have been amended for clarity in accordance with the Office's suggestions to obviate that stated objection. However, it is noted that a similar objection was applied to claims 39, 111, 131, and 139 (Office Action at page 3), which is believed to be in error. Whereas the "full complement"

of mutations present in JS cp45 may not correspond to the listed mutations set forth in claims 132, 134, 136, 140, 141, and 143, as asserted by the Office, claims 39, 111, 131, and 139 refer to exemplary recombinant viruses designated rcp45, rcp45 3'NCMFHN, rcp45 3'NL, rcp45 3'N, or rcp45 F, each of which has defined mutations provided in the specification, not JS cp45. Within the recombinant genome or antigenome of these recombinant viruses, defined mutations are incorporated which can be similarly incorporated into other recombinant viruses of the invention, regardless of the possible replacement of genes from other species of PIV.

Applicants have noted and corrected the error in claims 132, 134, 136, 140, 141, and 143 in reciting "i)...ii)...iii)...v)...vi)..." without including "iv".

The remaining issues raised by the Office relating to compliance with 35 U.S.C. 112, second paragraph are collectively obviated by a suggested deposit of the JS cp45 exemplary biologically derived mutant. Applicants disagree that such deposit may be required to conform the specification and claims with this section of the Act. Nonetheless, for the purpose of clarity and in order to advance the application to issuance, the JS cp45 vaccine candidate virus will be deposited in due course and in full compliance with the terms referenced at pages 5 and 6 of the Office Action.

Patentability Under 35 U.S.C. §§ 102 and 103

Claims 1-4, 6, 7, 10-17, 20, 21, 26, 27, 30, 33-40, 43, 44, 47-49, 52, 54, 56, 57, 59, 61-85, 88-91, 93, 94, 96-116, 118, 120-143 are rejected under 35 U.S.C. 102(e) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Belshe et al. (5,869,036). Applicants respectfully traverse the stated grounds for rejection and submit that the Belshe et al. reference neither teaches nor suggests the subject matter of the invention.

In setting forth the instant rejection, the Office concedes that Belshe et al. "does not provide a working example of the material set forth in applicant's claims." Instead, the rejections are founded primarily on the literal content of claims set forth in the Belshe et al. patent, which the Office relies upon as disclosure that is "legally presumed to be enabled".

The Office does not specifically identify the subject matter of the rejected claims that is thus considered anticipated, but merely asserts that "[f]or the materials claimed in the patent claims, the (Belshe et al.) patent anticipates the invention as claimed." For those materials "that differ from precisely what is claimed", the Office asserts that the Belshe et al. patent "explicitly suggests those materials."

As a preliminary matter, Applicants respectfully request clarification of the foregoing rejection with respect to 35 U.S.C. § 102. Whenever the Office relies upon an allegedly anticipatory reference to support a rejection under this section of the Act, the burden is initially on the Office to demonstrate that the reference discloses each and every element and limitation of the claimed invention.

> The factual determination of anticipation requires the disclosure in a single reference of every element of the claimed invention. . . .[I]t is incumbent upon the examiner to identify wherein each and every facet of the claimed invention is disclosed in the applied reference.

Ex Parte Levy, 17 USPQ2d, 1461, 1462 (Bd.Pat.App.Int. 1990) (emphasis supplied, citations omitted).

In the instant case, the general statements provided by the Office do not identify which claims are subject to rejection under 35 U.S.C. § 102, much less specify what subject matter within these claims is allegedly anticipated by the cited reference. In this regard, Applicants respectfully request clarification. Pending this clarification, Applicants reserve the right to submit evidence and remarks demonstrating differences in content and scope between the rejected claims and the claims presented in Belshe et al. However, such clarification of the rejection under 35 U.S.C. § 102 is likely obviated by the discussion below which demonstrates that the subject claims are not enabled by the disclosure of Belshe et al.

Concerning the latter issue, Applicants note that any reference relied upon by the Office as an anticipatory reference under 35 U.S.C. § 102 must fulfill all of the written description and enablement requirements of 35 U.S.C. § 112.

> The standard for anticipation by patenting is the same one of a full enabling disclosure that applies to printed publications, i.e., it must disclose the invention in such full, clear and exact terms as to enable any person skilled in the art to which the invention relates to practice it.

Electronucleonics Laboratories, Inc. et al. v. Abbot Laboratories, 214 USPQ 139, 147 (N.D. Ill. 1981) (underscore added, citations omitted).

As further explained by the Federal Circuit in *In re Donohue*, 226 USPQ 619, (Fed. Cir. 1985).

> It is well settled that prior art under 35 U.S.C. § 102(b) must sufficiently describe the claimed invention to have placed the public in possession of it.
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> [E]ven if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling. (emphasis supplied, citing In re Borst, 45 USPQ 544, 557 (CCPA 1965), *cert. den.* 382 U.S. 973, 148, USPQ 771 (1966).

In the instant case, the Belshe et al. reference provides neither a written description nor an enabling disclosure of the subject matter set forth in Applicants' claims—sufficient to place this subject matter into the hands of the public. For the same reasons, the Belshe et al. reference fails to render the instant claims obvious within the meaning of 35 U.S.C. § 103.

In contrast to Applicants' disclosure setting forth successful recovery of specific, attenuated recombinant PIV vaccine candidates, the Belshe reference contains no "working example" of any such materials or methods as are set forth in Applicants' claims—which is expressly conceded by the Office (Office Action at p. 6). On the contrary, Belshe et al. describe the simple ability of a plasmid expressing a wild type PIV3 L protein to enhance the replication of a JScp45 virus at a restrictive temperature of 39.5°C. These findings were offered as a basis for speculation by Belshe et al. that the L gene of cp45 possesses mutations that might be useful a recombinant PIV vaccine virus derived from cDNA. However, the virus recovered by Belshe et al. after complementation at the restrictive

temperature was not changed or modified in any manner contemplated by Applicants' disclosure. No cDNA constructs were designed and produced from which PIV3 wild type viruses could be recovered, and certainly no new constructs or recombinant viruses bearing specific, attenuating mutations were produced.

Resolving these deficiencies was critical to enablement of Applicants' claimed invention. At the same time, the absence of such disclosure in the Belshe et al. reference negates any "reasonable expectation for success" to achieve Applicants' invention. This is especially clear when the "particular results" achieved by Applicants are considered, namely that it was shown to be possible to construct recombinant PIV vaccine candidates from cDNA that are suitably attenuated, yet sufficiently immunogenic to produce a protective immune response in immunized hosts.

Examining the teachings of Belshe et al. in further detail, it is noteworthy that the subject mutations identified in the L gene of JS cp45 were described previously by Stokes et al. 1993 (of record). Likewise, the properties of JS cp45 as a candidate vaccine virus for primates were previously described by Hall et al. 1993 (of record). The independent contribution of the L gene mutations to the attenuation phenotype of PIV, or to the ts phenotype of the virus, are not described in the Belshe reference or elsewhere in the art of record. This is because the Belshe et al. specification fails to describe or enable recovery of any virus from cDNA, much less specific, attenuated PIV vaccine candidates as described in Applicants' specification.

The Belshe et al. reference speculates, but presents no findings, that a recombinant virus with one or more of the cp45 L mutations might serve as a useful vaccine candidate against PIV3. However, in order to determine whether such a recombinant virus contains the complex set of biological properties necessary for development of a live attenuated virus vaccine (including viability, attenuation, immunogenetic, and protective efficacy), it is first necessary to generate recombinant viruses using cDNA technology as disclosed by Applicants. It is further necessary to demonstrate that the phenotypic effect of any desired cp45 mutation, for example a ts mutation identified in L, can be segregated from

complementary or interactive effects of other cp45 mutations. Lastly, it is critical for validating the speculative teachings of Belshe et al. that any mutations thus identified and segregated into a viable recombinant vaccine candidate be attenuating and that such attenuation be balanced sufficiently to yield a protective immune response in susceptible hosts.

The simple complementation of replication for cp45 virus using a wild type L plasmid, as described by Belshe, was only conducted *in vitro* using tissue culture cells and was not validated by parallel studies *in vivo*. In this context, it was quite possible that recombinant virus incorporating one or more of the three "temperature sensitive" (ts) mutations in the cp45 L gene mutations would not be attenuating (att) *in vivo*. In particular, a finding that replication of cp45 may be complemented by wild type L protein in tissue culture cells is not clearly predictive that a virus bearing one or more of these mutations would be attenuated *in vivo*. This correlative deficiency is apparent from the following considerations.

As an initial point, it is known that entire classes of viruses called "temperature-dependent hose range (td-hr) mutants" are ts on one tissue but are not ts on other tissue culture cells. These td-hr mutants are not necessarily attenuated *in vivo* (see Snyder et al., Virus Research 15:69-84, 1990 and Shimizu et al., Virology 124:35-44, 1983—copy provided under separate cover for consideration and entry in the record). As described in Snyder et al., an exemplary mutant (clone 143-1) of influenza virus was shown to be highly ts in tissue culture cells, but was not significantly attenuated *in vivo*. Additional findings by Shimizu et al. indicate that such td-hr mutants are common and are found in many different complementation groups of the influenza virus (i.e., they are present in many different genes of the virus).

The Belshe et al. reference does not demonstrate whether any of the contemplated ts mutations in the L gene of cp45 belong in the td-hr class of mutations or in the other class of ts mutations whose replication is effected by the temperature present in the host animal. In view of this deficiency, the simple description of a complementation phenotype for a group of multiple, unsegregated mutations in a complete gene *in vitro* does not serve as a reliable indicator of attenuation *in vivo*.

As a second point relating to the reliability of teachings by Belshe et al., the reference also fails to describe specific levels of temperature sensitivity and/or attenuation for any virus bearing one or more of the three identified cp45 L gene mutations. These important properties cannot be reliably translated from an *in vitro* complementation system as described by Belshe et al. to a hypothetical recombinant PIV vaccine candidate *in vivo*. On the contrary, the teachings of the cited reference merely suggest that the cp45 mutations in L make some contribution to the ts phenotype *in vitro* in one cell line. From these limited teachings, it cannot be reliably predicted what level of temperature sensitivity and/or attenuation any recombinant virus with one or more of the L gene mutations of cp45 may exhibit. For example, if the contribution of the set of cp45 L mutations to the overall level of temperature sensitivity of cp45 was small, and a virus bearing all three cp45 mutations in L was restricted at 39.5°C (the only temperature tested by Belshe et al.) but not at 37°C, such a virus may not be attenuated at all in a host with a 37°C body temperature. Thus, the disclosure of Belshe et al. does not demonstrate any properties of the cp45 L gene mutations that are necessary for designing a recombinant virus that has useful properties for vaccine use.

The complex effects and interactions of mutations in the PIV3 cp45 virus that determine its level of temperature sensitivity and attenuation, as demonstrated in the present application, clearly show that predictions of *in vivo* properties of individual and collective mutations in recombinant PIVs cannot be reliably made based on *in vitro* complementation studies as presented by Belshe et al. For example, prior to Applicants' invention it was not predictable that the temperature sensitive phenotypes of the cp45 L gene mutations recovered in recombinant PIVs engineered from cDNA would not be not additive. However, as revealed in the instant specification, the assembly of cp45 992 and 1558 mutations generates a recombinant virus bearing two "ts" mutations that is <u>less</u> temperature sensitive than a recombinant bearing either single mutant. It is only with the benefit of Applicants' invention that these unexpected effects were discerned and recognized as useful tools for calibrating attenuation and immunogenicity in recombinant PIV vaccine candidates.

In relation to the foregoing points, the Belshe reference erroneously concludes that a temperature sensitive phenotype accurately predicts the presence of an attenuation

phenotype in a recombinant PIV. Applicants' disclosure reveals the flawed nature of this conclusion. In this context, the instant specification teaches that the rcp45 3'N recombinant was ts but not attenuated and, conversely, the rcp45 C and rcp45 F recombinants were attenuated and not ts. Furthermore, r942/992, which exhibited a level of temperature sensitivity comparable to that of cp45 virus, was overattenuated *in vivo*. Conversely, r992/1558 was much less attenuated that cp45. These unexpected findings underscore the deficiencies of the Belshe et al. patent— which fails to identify specific properties determined by individual cp45 L gene mutations, much less to reliably predict combinatorial phenotypes specified by sets of mutations incorporated within novel recombinant vaccine candidates and analyzed *in vivo*. Only through the use of Applicants' successful cDNA recovery system could these unexpected effects be determined and harnessed for use within the claimed methods and compositions.

Claims 5, 8, 9, 45, 46, 51, 53, 86, and 87 are rejected under 35 U.S.C. 103(a) as being unpatentable over Belshe et al. (Office Action at p. 7). In a related rejection (Office Action at p. 8), the Office considers that claim 41 is unpatentable over Belshe et al. as applied to claims 5, 8, 9, 45, 46, 86, and 87, and further in view of Karron et al. (J. Infect. Dis. 171:1107-1114, 1995). Applicants respectfully traverse.

The examiner suggests that the Belshe patent teaches hybrid viruses, for example recombinant viruses bearing heterologous proteins such as protective antigens and mutated proteins of one PIV or non-PIV virus substituted for those of a recipient PIV virus. Applicants respectfully submit that the teachings of Belshe et al. are deficient and that the subject matter of the rejected claims is patentable thereover, for the reasons noted above. The teachings of Karron et al., limited to bovine PIV, do not remedy these deficiencies. Further, the Office is urged to consider related findings from negative strand viruses as well as those from other families of viruses that indicate that chimerization, particularly involving the substitution of one virion glycoprotein for its functional counterpart in a different virus, is expected to yield complex and unpredictable phenotypic effects. For example, a recombinant measles virus in which the HA and F proteins of measles virus were replaced by the G protein of VSV was highly attenuated in replication *in vitro* making it extremely difficult to produce

such a virus for use as a live attenuated vaccine (Spielhofer et al., J. Virol. 72:2150-2159, 1998— copy provided under separate cover for consideration and entry in the record). A second example of the attenuating effect of chimerization is seen in which the genes encoding the virion proteins of tick-borne encephalitis virus, a flavivirus, were hybridized to a dengue virus backbone (Pletnev et al., Proc. Natl. Acad. Sci. USA 89:10532-10536, 1992— copy provided under separate cover for consideration and entry in the record). The resulting hybrid virus was highly attenuated for mice compared to itsTBE parent virus.

Belshe et al. specifically teaches that chimerization in this context would be neutral in terms of replication of the hybrid virus. However, the cited reference and other art of record does not provide an adequate basis for this prediction. On the contrary, in view of the foregoing facts the effects of chimerization on replication *in vitro* or *in vio* is unpredictable without specific data such as the results disclosed in Applicants' specification teaching, e.g., a viable PIV1-PIV3 chimera.

Claims 18, 19, 28 and 29 are rejected under 35 U.S.C. 102(e) as anticipated by Belshe at al., or, in the alternative, under 35 U.S.C. 103(a) as obvious over Belshe et al. in view of Stokes et al. (Virus Research 30:43-52, 1993). Applicants respectfully traverse. In this regard it is submitted that the teachings of Belshe et al. are deficient and that the subject matter of the rejected claims is patentable thereover, for the reasons noted above. Further, there is no additional disclosure in the Belshe et al. or Stokes et al. references that would render recombinant RSV having specific substitutions in the N protein enabled or provide a reasonable expectation of success for achieving such recombinants having the characteristics disclosed by Applicants.

Claims 22-25, 31, 32, 42, 60, 117, and 119 are rejected under 35 U.S.C. 103(a) as being unpatentable over Belshe et al. in view of Conzelmann (J. Gen. Virol. 77:381-389, 1996). The teachings of Belshe et al. are deficient and the subject matter of the rejected claims is patentable thereover, for the reasons noted above. The general review by Conzelman does not add to the disclosure of Belshe so as to enable the subject claims or provide a reasonable

expectation of success for achieving the claimed subject matter and results disclosed by Applicants.

Claims 11, 48, 50, 52, 55, 56, 58, 91 and 92 are rejected under 35 U.S.C. 102(b) as being anticipated by Dimock et al. (J. Virol. 67:2772-2778, 1993). Applicants respectfully traverse.

The Dimock et al. reference discloses a limited, minigenome system for recovering synthetic "analogs" of genomic RNA and replicative-intermediate RNA of HPIV3. The reference clearly does not disclose the polynucleotide of claim 1 encoding a PIV genome or antigenome as recited. Nor does the reference disclose any of the compositions of claims 48 and 50, methods of claims 52, 55, 56, and 58 or infectious PIV particles of claims 91 and 92—which involve admixture, coexpression or association of a PIV genome or antigenome and N, P, and L proteins to produce an infectious PIV particle. The rational offered by the Office to purportedly equate this subject matter with the disclosure of Dimock et al. is founded on a misinterpretation that the term "expression vector" of claim 52 "encompasses PIV itself." This is not intended by the recited language and the comments provided above in the section pertaining to Patentability Under 35 U.S.C. § 112 fully clarifies the record in this regard. Apart from this issue, Dimock et al. fail to disclose admixture, coexpression or association of a PIV genome or antigenome and N, P, and L proteins to produce an infectious PIV particle. Thus, irrespective of whether the term "PIV" encompasses "subviral particle", the so-called "defective PIV" of Dimock et al. does not read on the subject matter of the rejected claims.

Claims 11, 48, 49, 52, 54, 57-59, 60, 91 and 93 are rejected under 35 U.S.C. 102(a) as being anticipated by Kato et al. (Genes to Cells 1:569-579, 1996). Applicants respectfully traverse. However, the merits of the rejection are not addressed herein in view of the amendments above presented for clarification and to advance certain aspects of the invention to issuance. These amendments obviate the rejection by clarifying that claims 11, 48, 49, 52, 54, 57-59, 60, 91 and 93 are directed to polynucleotides, recombinant PIVs and particles encoding or incorporating a "human or bovine PIV genome or antigenome."

In further reviewing the Kato et al. reference and remaining art of record, Applicants urge the Office to consider yet additional deficiencies of these references relating to unique aspects of PIV recovery systems. In particular, the PIV rescue system disclosed by Applicants departs in fundamental and significant aspects from other rescue systems for negative stranded RNA viruses, including for example rabies and VSV rescue systems. At the same time, the human PIV3 rescue system disclosed by Applicants differs substantially from the murine PIV (Sendai) virus recovery system reported by Kato et al.

An important aspect of Applicants' PIV3 rescue system that is not disclosed or suggested by the cited references is the distinct organization of the P gene. The P gene of human PIV3 differs from that of all other systems described in the art or record. In particular, the HPIV3 P gene contains ORFs that can encode four distinct proteins, P, C, D, and V. In contrast, VSV and rabies only encode one P-related protein. Sendai virus encodes a P protein, several C proteins that are carboxy co-terminal, and a V protein. None of these other viruses contain an ORF related to the PIV3 D protein. Since prior rescue systems required the expression of L, N and P proteins, it was unknown at the time of the invention whether successful HPIV3 recovery would require expression of the D ORF as disclosed by Applicants in construction of the pTM(P) expression plasmid. The complex roles of proteins other than N, P and L for negative stranded RNA viral was recently underscored for RSV, wherein it was found that expression of the M2-2 protein was required for successful recovery of RSV from cDNA (Collins et al., Proc. Natl. Acad. Sci. USA 92:11563-67, 1995, of record).

Yet another obstacle to successful recovery of human and bovine PIV related to the critical need for sequence fidelity in constructing components of a rescue system. Sequences determined for cloned cDNAs of various RNA viruses were shown to contain frequent errors, due to several factors. One identified factor was a high error rate and lack of proof-reading of the polymerases of RNA viruses (Steinhauer et al., Gene 122:281-8, 1992, copy will be provided to the Office if so requested). In the absence of proof-reading, the misincorporation rate is thought to be as high as 10^{-3} to 10^{-4} (Steinhauer et al., J. Virol. 63:2063-2071, 1989, copy will be provided to the Office if so requested). Thus, for a genome

of 15,000 nucleotides, each molecule would have between 1.5 and 15 changes relative to the consensus.

The vast majority of changes that result in amino acid changes or changes in cis-acting signals would be expected to be deleterious. RNA viruses have long been known to have high polymerase error rates and heterogeneous populations (Batschelet et al., Gene 1:27-32, 1976; Domingo et al., Cell 13:735-744, 1978, copies will be provided to the Office if so requested). Viruses bearing deleterious mutations are readily generated and propagated under the standard *in vitro* passage conditions needed to achieve high titers. However, when virus is passaged under permissive conditions with the use of plaque isolation to eliminate nonfunctional mutants, the genomic sequence is surprisingly stable. This is an indication that the genomic sequence is probably "optimal" at many critical positions and does not readily tolerate most mutations.

Another source of error in cDNAs is the reverse transcription step, which involves a polymerase that also does not have a proofreading function and hence has an error rate similar to that mentioned above. If the DNA is amplified by PCR, this provides another source of error. Although thermostable polymerases that are capable of proof reading and thus possess a lower error rate are now available, this gain in fidelity is partly offset by the multiple rounds of amplification involved in PCR, since each round provides an opportunity for additional errors. Finally, cloning and propagation in bacteria can be a source of mutation.

Consistent with the foregoing discussion, numerous examples of mutations or sequencing errors in sequenced cDNAs of nonsegmented negative strand RNA viruses have been identified. Some examples are summarized below (copies of cited references will be provided to the Office if the Examiner so requests):

i) Sequence analysis of the vesicular stomatitis virus (VSV) L gene identified a large number of differences between ostensibly-identical cDNAs, indicative of many mutations. Indeed, this large number of mutations was reflected in the title of the article: "Primary structure of the vesicular stomatitis virus polymerase (L) gene: evidence for a high frequency of mutations" (Schubert et al., J. Virol. 51:505-514, 1984). Considerable effort was

needed to assemble and identify a VSV L gene which appeared to be functional (Schubert et al., Proc. Natl. Acad. Sci. USA 82:7984-7988, 1985).

ii) Wertz et al. (Patent 5,789,229, of record) described that their initial full length RSV L cDNA clone contained an error that resulted in a prematurely-terminated product due to a frame shift.

iii) Wertz et al. (ibid) describe a complete sequence for an RSV L cDNA. However, compared to the sequence of recovered recombinant RSV (Collins et al., Proc. Natl. Acad. Sci. USA 92:11563-11567, 1995, of record), that of Wertz et al. has an A to G nucleotide change at L gene position 57. This results in a serine 17 to glycine amino acid change in the L protein. This mutant L protein was competent to direct minigenome RNA replication (Yu et al., J. Virol. 69:2412-2419, 1995), but its ability to direct transcription and other polymerase functions was not described. The RSV M2-1 and L genes overlap, and the gene-end signal of the M2-1 gene is contained at positions 57 to 68 (Collins et al., Proc. Natl. Acad. Sci. USA 84:5134-5138, 1987, of record). Thus, the change at position 57 alters the first nucleotide of this signal, which is part of a motif that is conserved in all of the human (subgroup A and B), bovine and ovine RSVs sequenced to date. Thus, if this sequence is attempted to be included into infections virus, the possibility exists that infectivity would be compromised by (1) the amino acid change in the L protein, which might have a deleterious effect not evident in the simple replication assay used by Wertz et al., *supra*, and (2) the nucleotide change in the M2 gene-end signal, which might reduce the efficiency of its activity, which would have the effect of increasing readthrough transcription into the L gene and decreasing the synthesis of monocistronic L mRNA and L protein, which would be expected to be deleterious.

iv) Grosfeld et al., J. Virol. 69:5677-5686, 1995 described another RSV L cDNA that was found to have a single D989N point mutation which rendered it completely inactive.

v) The original sequence of the Sendai virus L gene provided evidence of multiple open reading frames that turned out to be due to mutations or sequencing errors (Morgan and Rakestraw, Virology 154: 31-40, 1986).

vi) The original sequence published for the RSV N gene turned out to be an artifactual chimera between the N and G genes (Elango and Venkatesan, Nucleic Acids Res. 11:5941-5951, 1983; Collins et al., Virology 146:69-77, 1985).

vii) The original sequence of the VSV P protein (then called NS) was later found to be prematurely terminated by 43 amino acids (Gallione et al., J. Virol. 39:529-535, 1981; Hudson et al., J. Gen. Virol. 67:1571-1579, 1986).

viii) The original L sequence of Marburg virus (Muhlberger et al., Virology 187:534-547, 1992) contained numerous errors, including reading frame shifts (A Bukreyev, unpublished data).

ix) The original published sequence for the RSV F gene (Collins et al., Proc. Natl. Acad. Sci. USA 81:7683-7687, 1984) turned out to have four amino acid changes compared to a functional clone, even though parts of the original sequence had been obtained from more than one cDNA (Anderson et al., J. Gen. Virol. 73:1177-1188, 1992).

x) The original published sequences of the Sendai virus F protein and the human parainfluenza virus 3 (HPIV3) F protein each had at least one mutation leading to the deletion of an N-linked glycosylation site (Blumberg et al., J. Gen. Virology 66:317-331, 1985; Spriggs et al., Virology 152:241-251, 1986).

xi) Papers describing sequence analysis of several RSV genes documented heterogeneity among individual cDNAs (Collins et al., Proc. Natl. Acad. Sci. USA 81:7683-7687, 1984; Collins and Wertz, Virology 141:283-291, 1985; Wertz et al., Proc. Natl. Acad. Sci. USA 82:4075-4079, 1985; Stec et al., Virology 183:273-287, 1991).

xii) The original sequences derived for the HPIV3 JS parent and its cp45 derivative each contained at least one mutation or sequencing error (Stokes et al., Virus Res.

25:91-103, 1992; Stokes et al., Virus Res. 30:43-52, 1993). In the case of the cp45 virus, this resulted in an initial failure to identify several cp45 mutations (Skiadopoulos et al., J. Virol. 73:1374-1381, 1999).

xiii) The original sequence determined for the cold passage (cp) RSV virus that is the progenitor of a series of biologically-derived and recombinant vaccine candidates contained a cDNA cloning or sequencing error that resulted in the initial failure to identify a cp mutation (Connors et al., Virology 208:478-484, 1995).

From the foregoing evidence it is clear that there are numerous potential sources of errors in developing cDNAs for use in the production of recombinant PIV vaccine viruses. To fully appreciate the importance of such errors, it is necessary to consider that the genome of HPIV3 contains 15,462 nucleotides. The support plasmids that are necessary for recovery of PIV3 include cDNAs with an aggregate length of more than 10,000 nucleotides. Thus, there is a high expectation of significant errors that is necessary to overcome in order to recover HPIV3.

To overcome the various problems that attend sequencing errors, and to otherwise facilitate development of a cDNA-based recovery system for negative stranded RNA viruses, it is possible to develop assays that test the functionality of individual ORFs, directly or indirectly. For example, an indirect assay in the case of a glycoprotein may involve evaluation of its intracellular folding and processing. A direct assay may be to reconstruct fusion in cell culture using coexpressed cDNAs. A functional assay for nucleocapsid/polymerase proteins might utilize a minigenome system. However, many proteins lack such assays, either because they have not yet been devised or because the functions of subject proteins remain unknown. Also, it should be noted that these assay systems are complex and challenging to construct, as evinced by the fact that such achievements are typically reported in full-length research papers and by presentation at international conferences. Furthermore, these assays can detect functionality of an ORF, but typically do not provide additional, qualitative or quantitative information regarding the

expression of a subject ORF. In addition, proteins can have additional functions that are either unknown or untestable.

In summary, only the recovery of specific, full length cDNA sequences into viable recombinant viruses, followed by demonstration that the recombinant virus is unimpaired for growth and infectivity, can identify a "correct" sequence for recombinant viral genomes and encoded proteins for use within the invention. However, the initial development of a successful viral recovery system itself requires a high level of fidelity for this prerequisite sequence information, which fidelity is clearly shown by the above references to be unpredictable. On this basis it is respectfully submitted that the foundational cDNA recovery system for PIV, first developed by Applicants, is neither taught nor suggested by the art of record within the meaning of 35 U.S.C. §§ 102 or 103.

CONCLUSION

In view of the foregoing, Applicants believe that all of the claims now presented for review in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is thus respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Date: June 19, 2000

By: _____
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